PHYTOCHEMICAL INVESTIGATION, HEPATOPROTECTIVE ACTIVITY, ANALGESIC ACTIVITY AND ANTIMICROBIAL ACTIVITY OF AERIAL PARTS OF *POLYGONUM GLABRUM* WILLD

Dissertation

Submitted in partial fulfillment of the requirement for the

award of the degree of

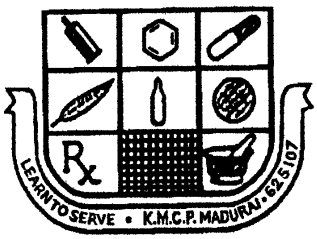
MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI.



DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

K.M.COLLEGE OF PHARMACY

UTHANGUDI, MADURAI - 625 107

APRIL – 2012

CERTIFICATE

This is to certify that the dissertation entitled "**PHYTOCHEMICAL INVESTIGATION, HEPATOPROTECTIVE ACTIVITY, ANALGESIC ACTIVITY AND ANTIMICROBIAL ACTIVITY OF AERIAL PARTS OF *POLYGONUM GLABRUM* WILLD**" submitted by **Mr**. **JUNO RAPHAEL** to The Tamilnadu Dr.M.G.R.Medical University, Chennai, in partial fulfillment for the award of Master of Pharmacy in Pharmaceutical chemistry at K.M. College of Pharmacy, Madurai, is a bonafide work carried out by him under my guidance and supervision during the academic year **2011-2012.**

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DEDICATED TO MY

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ACKNOWLEDGEMENT

With deep sense of gratitude and veneration I express my profound sense of appreciation and love to my beloved father ***Mr. M. M. Raphael*** *and my mother* ***Mrs. Anna Raphael*** *for providing me love like heaven's caring arms both materially and emotionally.*

It is an honor to pay my respect and heartfelt thanks to our most respected correspondent ***Prof. M. Nagarajan M.Pharm., M.B.A., DMS (IM), DMS (BM),*** *K.M.College of pharmacy, Uthangudi, Madurai, for providing necessary facilities to carry out this dissertation work successfully.*

I take this opportunity with pride and immense pleasure in expressing my deep sense of gratitude to beloved guide ***Mrs. R. Meera, M.Pharm.,*** *Assit.Professor, Department of Pharmaceutical Chemistry for her valuable guidance. Her impressive, innovative ideas and constructive suggestions have made the presentation of my work a grand success.*

My sincere gratitude to ***Dr. S. Jayaprakash, M.Pharm., Ph.D.,*** *Principal, & Head, Department of Pharmaceutics, K.M.College of Pharmacy, Uthangudi, Madurai, for his most valued suggestions and encouragement during the course of study.*

I would like to express my profound sense of gratitude to ***Dr.S.Venkataraman, M.Pharm., Ph.D.,*** *Professor and Head, Department of Pharmaceutical Chemistry for his valuable suggestions and help throughout this work.*

With deep sense of veneration and gratitude I am really indebted to ***Mr.J.Raamamurthy, M.Pharm.,*** *and* ***Mr. P. Muthumani, M.Pharm.,*** *professors, Department of Pharmaceutical Chemistry for their motivation, suggestion and constant encouragement during my work.*

I express my heartfelt thanks to ***Mrs. P. Devi, M.Pharm., (Ph.D)*** *Department of Pharmacognosy, for her valuable help during my work.*

I am deeply indebted to ***Mr.N.Chidambaranathan, M.Pharm.,*** *Asst.Professor, Department of Pharmacology for his immeasurable esteemed help in carrying out the pharmacological studies.*

My sincere thanks to ***Dr. D.Stephen*** *(Head, department of Botany, American college, Madurai) for his help in identification and authentification of plant.*

I owe my humble thanks to ***Prof. M. S. Prakash, M.Pharm.,*** *and* ***Dr.Meena, M.Pharm., Ph.D.,*** *Department of Pharmaceutical Analysis to their constant help in completing this project work.*

. *My sincere thanks to my seniors* ***Mr. Anoop Thomas, M.pharm.,*** *and* ***Mr.S.Sivaraj, M.Pharm.,*** *for their valuable advises and suggestions to complete my project work.*

I sincerely thank ***Miss.A.Vijayalakshmi,*** *Lab Assistant, and* ***Mrs.Shanmugapriya****, store in charge for their gracious render able help during my project work.*

I extend my thanks to all teaching and Non-teaching staffs of K.M.college of Pharmacy for their help.

With deep sense of affection I express my heartfelt gratitude to my classmates ***Mr.M.Rajaraman, Mr.M.Jagadheeswaran, Miss.Vidya Ramachandran, Mrs.Swathy Sairam*** *and my close friends* ***Mr.Anoop .P, Mr.R.Vivek Chathrapathy, Mr.Mathew Ebin Sovichan****, and my juniors* ***Mr.Ajith.K.S, Mr.Leo Lawrense****.*

Juno Raphael

CONTENTS

RESULTS AND DISCUSSIONS

Several chemical constituents and physiochemical properties of *Polygonum glabrum* willd, I have decided to work on the aerial part, since it's widely used in the indigenous medicine. The dried aerial parts were made in to a coarse powder and extracted with **Pet. ether AR, Chloroform AR, and Methanol AR**. Solvents in the order of increasing polarity.

The physiochemical screening of *Polygonum glabrum willd* was to study, isolate and characterization of chemical constituents and pharmacological activity. During my investigation the above mentioned solvents, extracts showed the presence of **carbohydrate, glycoside, flavonoids, flavones, proteins and alkaloid.**

Regarding the isolation procedure is concerned the chloroform extract was subjected to column chromatography using the silica gel 100-200 mesh. During my investigation three compounds were isolated namely **PG 1, PG 2, PG 3**. These compounds were eluted with the solvents in the increasing order of polarity like Pet ether, benzene, chloroform, ethyl acetate and methanol.

The compound **PG 1** showed green colour which is semisolid in state. The melting point was 200-220ºc which is soluble in absolute alcohol and chloroform. The TLC showed a single spot using ethyl acetate : hexane (6:4) having the UV absorbance of 270 to nm. The R_f value was found to be 0.72. The IR data showed the frequency at 3345.50, 2921.69, 1651.70, 1541.71, 673.35 cm^{-1} and ^{1}HNMR showed the signals at 0.896, 1.233, 2.3328, 7.398 δppm showed that this compound may be **flavonoids** type, which was confirmed by chemical test.

The compound **PG 2** showed brown colour which is semisolid in state. The melting point was 170-180ºc which is soluble in absolute alcohol and chloroform. The TLC showed a single spot using ethyl acetate : hexane (4:6). The R_f value was found to be 0.78. The IR data showed the frequency at 3447.99, 2919.70, 1630.25, 1454.54, 1163.63, 675.09 cm^{-1} and ^{1}HNMR showed the

signals at 0.827, 1.568, 3.688, 7.262 δppm. This suggested that this compound may be **Flavonoids** type, which was confirmed by chemical test.

The compound **PG 3** showed yellowish brown in colour which is semisolid in state. The melting point was 160-170ºc which is soluble in absolute alcohol and double chloroform. The TLC showed the single spot using hexane : ethyl acetate (7:3), R_f value was found to be 0.75. The IR data showed the frequency at 3421.94, 2919.70, 1560.36, 1426.57, 1211.18, 665.73 cm^{-1} and ^{1}HNMR showed the signals at 0.863, 1.284, 3.877, 7.262 δppm suggested that these compound may be a **Flavonoids** type, which was confirmed by chemical test..

Regarding hepatoprotective activity, the rat groups G4, G5 administrated with *Polygonum glabrum* at a dose 200 and 400mg/kg respectively showed a significant decrease in the serum AST, ALT, ALP levels and a significant increase in serum total protein and total albumin levels. This was most likely due to the anti oxidant effect of P*olygonum glabrum* constituents. While in G5 high dose *Polygonum glabrum* showed a significant recovery towards normal, this result showed hepatoprotection after a both dose of *Polygonum glabrum extract* in experimentally drug induced hepatitis (DIH) in rats.

As for as the analgesic activity is concerned, the methanolic extract of *Polygonum glabrum* exhibited significant analgesic activity than petroleum ether extract.

Microbiological screening reveals that methanolic extract posses significant antimicrobial activity when compared with pet. Ether extract.

CONCLUSION

Since a number of phyto constituents are present in these extracts, further studies were planned to isolate and characterize each and individual compounds.

During the study of hepatoprotective activity, the methanolic extract has a significant protective action against the hepatotoxicity induced by the drugs used in the treatment of tuberculosis. The hepatoprotective role of extract might be due to its antioxidant potential mechanism suggesting that the extract of plant may be useful to prevent the oxidative stress induced damage.

Analgesic activity showed methanolic extract possesses significant activity when compared to pet. Ether extract.

As regards microbiological evaluation methanol extract revealed significant activity against Staphylococcus aureus, Pseudomonas aeurogonosa, Candida albicans when compared to pet. Ether.

The natural and synthetic phyto constituents play a vital and a major role in pharmacological, clinical studies, which showed an important impact in preparing natural product based libraries for combinational chemistry.

Henceforth, at docimasy, these therefore provide a basis for detailed investigation of various parameters on therapeutic efficacy, which undergo to obtain excellent use in indigenous medicine.

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PICTURE OF POLYGONUM GLABRUM

INTRODUCTION[1-10]

20th century has witnessed much attention directed to synthetic compounds and the rise of combinatorial chemistry as an important part of the drug discovery process. However, with the turn of the century much interest has returned to the natural compounds for drug discovery. This is mainly due to the fact that natural products generally have a better safety profile and also have a so-called privileged structure. This is because compounds produced by living organisms have evolved over millennia and is therefore more likely to have a specific biological activity, rather than "randomly" assembled, man-made synthetic chemicals.[1]

The term natural products refers to secondary metabolites, small molecules (mol.wt. < 2000 amu) produced by an organism and these metabolites are not strictly necessary for the survival of the organism. Natural products include: (1) an entire organism, (2) part of an organism, (3) an extract of an organism or part of an organism and exudates and (4) pure compounds (e.g. alkaloids, coumarins, flavonoids, glycosides, xanthones, lignans, phenylpropanoids, isoprenoids, sugars, etc.) isolated from plants, animals or microorganisms. Many of these compounds show a variety of biological and pharmacological activities and some of these compounds are essential for everyday life, both for humans and animals.[2]

The medicinal chemicals used throughout the world were isolated from natural sources. These sources include flowering plants, fungi, bacteria and to lesser extent animals, especially marine animals. The subfield of organic chemistry that deals with isolating and studying chemicals found in nature is called natural product chemistry.[3]

Chemistry of natural products involves characterization of their composition and isolation of specific active components with different techniques, which lie in various fields of science specially chemistry.[1]

As a result, in last three decades, we have identified various new classes of potential pharmacophores against various diseases. Different clinically important

enzymes were targeted such as α-glucosidase, thymidine phoshphorylase, acetylcholinesterase, butyrylcholinesterase, glucuronidase, phosphodiesterase, tyrosinase, which led to the discovery of potent and novel pharmacophores. Along with this, a battery of *invitro* and *in-vivo* bioassays were employed to identify new antibacterial, antifungal, antiparasitic, antioxidant, antiangiogenic and antiglycation agents.

However, the field of phytopharmaceutical research and development is now witnessing a major transformation both in terms of concept and in practices. With the advent of super-advanced hyphenated techniques such as LC-NMR, LC-MS, LCMS/NMR, GC-MS, cryo- and nanoprobes, etc, new spectroscopic methods and high throughput bioassay techniques, the research in plant-based drug discovery has immensely progressed in recent years. The emerging new field of metabolimics and associated technological advancements also holds great promises for the future of this exciting discipline.[4]

At present there are many chronically debilitating or life-threatening diseases that urgently require improved or new medical treatments. Chemotherapy is one of the well-established approaches for their control. With new diseases and increasing resistance to existing drugs, there is a pressing need to discover and develop new innovative drugs with diminished side-effects to combat cancer, viruses and other threats. The search for anti-cancer agents from plants started in the 1950s with the discovery and development of vinblastine and vincristine, the so-called vinca alkaloids, and the isolation of the podophyllotoxins.

In 1960, the United States National Cancer Institute (NCI) initiated an extensive plant collection program leading to the discovery of numerous novel cytotoxic agents, including the taxanes and camptothecins. With the leads from natural products, many anti-cancer drugs have been developed.[5]

The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary

health care needs. Also, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side-effects, easily available at affordable prices and sometime the only source of health care available to the poor.[6]

The rapid growth in popularity of herbal and plant medicines raises some concerns Herbal medicine is extremely varied from place to place, making it very difficult to standardize safety regulations and assessment. Countries have very different regulatory laws in place for traditional forms of medicine, and some countries may not impose any regulation at all. Therefore, it is important to be knowledgeable about the quality and origin of any herb or plant used medicinally. Additionally, consumers should keep sustainability in mind; some medicinal herbs and plants are taken from the wild rather than cultivated, putting them in danger of over-harvesting. Finally, because medicinal herbs are natural, people may believe they hold no risk. Many medicinal plants do have negative side effects if dosing or administration instructions are not followed, and herbal treatments should always be undertaken in consultation with a doctor.[7]

Medicinal plant sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of India. India has one of the richest plant medical cultures in the world. According to an all India ethino biological survey carried out by the Ministry of Environment & Forests, Government of India, there are over 8000 species of plants being used by the people of India.There are estimated to be around 25000 effective plant based formulations used in folk medicine and known to rural communities all over India and around 10000 designed formulations are available in the indigenous medical texts. Around 70% of India's medicinal plants are found in tropical areas mostly in the various forest types spread across the Western and Eastern Ghats, the Vindhyas, Chotta Nagpur plateau, Aravalis & Himalayas.[6]

The turnover of herbal medicines in India as over the counter products, ethical and classical formulations and have remedies of Ayurveda, Unani and Siddha systems of

medicine is about $1 billion with a meager export of $ 80 million. 80% of the exports to developed countries are of crude drugs and not finished formulations leading to low revenue for the country. The list of medicinal plants exported from India are Aconitum species (root) Acorus *calamus* (rhizome), *Adatoda vasica* (whole plant), *Berberis aristata* (root), Cassia *augustifolia* (leaf and pod), *Colchicum luteum* (rhizome and seed), *Hedychium spicatum* (rhizome), *Heradeum candicans* (rhizome), *Inuia racemose* (rhizome), *Juglans reya* (husk), *Juniperus conimunis* (fruit), *Juniperus macropoda* (fruit), *Picrorhizn kurroon* '(root), Plantago ovata (seed and husk), *Podophyllum emodi* (rhizome), *Pinicn. yanatum* (flower, root and bark), *Rauwolfia serpentina* (root). Rheum *emodi* (rhizome), Saussurea (rhizome), *Swertia shirayita* (whole plant), *Valerian-intamansi* (rhizome), Zingiber *officinale* (rhizome) Five of these, i.e. Glycerrhiza glabra, *Commiphora mukut,* Plantago *ovata. Aloe barbadensis* and *Azardica indica* are used in modern medicine. Others are used in 52 to 141 herbal formulations and Triphala (Terminalia *chebula,* and *Emblica officinalis)* along is used in 219 formulation.

Liver, the largest gland is the metabolic "engine room of the body". Almost all the drugs, foods and water constituents are metabolized and detoxified in the liver. So it is often exposed to maladies resulting in a number of syndromes. Liver diseases mainly classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). The main causes for the liver disorder are by toxic chemicals (certain antibiotics, peroxidised oil, chlorinated hydrocarbon), excess consumption of alcohol, infections and auto immune disorders.

The liver dysfunction remains as one of the serious health problem but we donot have satisfactory antihepatotoxic drugs in the allopathic medicinal practice for serious liver diseases. However, a number of plants have shown to possess hepatoprotective properties by improving the antioxidant status. Yet, there is lack of scientific proofs to authenticate the hepatoprotective properties of those plants which are used traditionally to treat liver diseases. In recent times, therefore, the focus on the plant research has increased all over the world and large body of evidence has collected to show immense

potential of medicinal plants used, and hence the importance of natural products for drug discovery has been found impressive.[8]

Polygonaceae, the buckwheat or knotweed family is a cosmopolitan family, geographically distributed from the tropics to the arctic region and it is a family of dicots containing approximately 1,000 species in 30 genera. The name of the family is derived from Greek word "*Polygonon*", "Polys" meaning many and "gonon" meaning Knee referring to the prominent nodes of many species. Members of the Polygonaceae are diverse in habit ranging from annual or perennial herbs, shrubs to lianas and some trees. The plant polygonum glabrum comes under the genus polygonum, commonly known as common marsh buckwheat. The plant distributed throughout india, particularly in marshy places.[9]

The future of phytopharmaceuticals is bright as it undoubtedly serves as a cheap and steady for varied of therapeutic agents which are of great significance in the health care of mankind. In general, natural drug substances offer vital and appreciable roles in the modern system of medicine thereby adequately justifying their legitimate presence in the prevailing therapeutic arsenal.[10]

REVIEW OF LITERATURE[11-30]

B. Ezhilan et al.,[11] studied on the active principles in the whole plant ethanolic extract of *Polygonum glabrum* by GC-MS analysis clearly showed the presence of six compounds, the ether compound –Propane 1,1-diethoxy, alkane compound -2-Heptane, 5-ethyl-2,4-dimethyl, sulphur compound –Tiophene-2-Carboxamide, N-(2-furfuryl), alcoholic compound -1,14-Tetradecanediol, and plasticizer compounds -1,2-Benzenedicarboxylic acid, isodecyloctyl ester and 1,2,3-Benzenetriol . The results of this study offer a base of using *Polygonum glabrum* as herbal alternative for the synthesis of antimicrobial agents.

Intisar Sirour Mohammed[12] conducted phytochemical studies of flavonoids from *polygonum glabrum*. The fractionation of flavonoids was successively done with preparative thin layer chromatographic techniques. Two compounds were obtained in pure form (yellow crystals) and identified as being flavonoids.

Ulla Jacsson et al.,[13] isolated four biologically active diesters of 2,3-dihydroxyisodrimeninol from the leaves of *Polygonum glabrum***,** which used as an anthelmintic agent in the traditional medicine of Sudan. Since the new compounds differed only in one of the acid residues, the structural elucidation was carried out essentially on one of the compounds, using 1D and 2D NMR techniques as well as mass spectroscopy (EI+CI).

Khadiga Elnimir et al.,[14] conduct assessment of invitro antimalarial activity of *polygonum glabrum* and *Tamarindus indica* against *Plasmodium* faliciprum. The *in vitro* test of the ethanolic extract of *Polygonum glabrum* against Plasmodium falciparum showed mean difference at the (0.01 level) and IC50 (6.6µg/ml) with significant effect. While *Tamarindus indica* showed mean difference at the (1.00 level) with no significant effect.

Phytochemical studies indicated that the isolated crystal from crude ethanolic extract of the leaves of *P. glabrum* had a character of flavonoids. Yellow crystal was nearly similar to that of quercitrin (quercetin-3-LRhamnoside). The crystal m.p. was found to be 178Cº. Toxicological studies on *T. indica* and *P. glabrum* showed no

significant effect, however *P. glabrum* produced significant increase in serum-creatinine that suggests changes in renal function.

Queen Rosary Sheela et al.,[15] isolated and characterized two flavanone from the leaf *extract of Polygonum barbatum*

Compound **1** was identified as 5, 7, 2', 5'-tetrahydroxy-6-methoxyflavanone. A molecular formula of $C_{16}H_{14}O_7$ [(M+ at m/z 318, mp 120-122 °C)] was isolated as colorless crystals. Compound **2** was obtained as pale yellow powder was identified as 7-methoxy-3, 5, 8-trihydroxyflavanone. A molecular formula of $C_{16}H_{14}O_6$ [(M+H+) peak at m/z 303.0869, mp 222.6-228 °C] was assigned for the compound.

M. Abdul Mazid et al.,[16] isolated 3 compounds from the aerial parts of *polygonum barbatum* by using a combination of the normal phase column chromatography and preparative thinlayer chromatography on silica gel. The compounds were sitosterone from the petroleum ether fraction, and viscozulenic acid and acetophenone from the chloroform fraction of the methanol extract. The free-radical-scavenging properties of the isolated compounds were evaluated by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. None of the compounds showed any significant free-radical-scavenging activity in the DPPH assay.

Helena D. Smolarz et al.,[17] isolated two acetylated flavonoid glycosides: quercetin-3-*O*-b-D-(6≤-*O*-galloyl)-glucopyranooside and quercetin-3-*O*-b-D-(6≤-*O*-galloyl)-galactopyranoside together with quercetin-3-*O*-b-D-glucuronide, quercetin-3-*O*-b-D-glucopyranoside, quercetin-3-*O*-b-D-galactopyranoside, quercetin-3-*O*-a-Lrhamnpyranoside, quercetin-3-*O*-rhamnoglucoside, kaempferol-3-*O*-D-glucopyranoside, quercetin, kaempferol and taxifolin, from the aerial parts of *Polygonum lapathifoluim* ssp *tomentosum* (Schrank) Dans.

Hediat et al.,[18] studied on the antimicrobial activity and Phytochemical analyses of *polygonum aviculare*. Phytochemical studies revealed the presence of tannins, saponins, flavonoids, alkaloids and sesquiterpenes. The chloroform extract gave very good and excellent anti microbial activity against all tested bacteria and good activity against all tested fungi. Structural spectroscopic analysis that was carried out on the

active substances in the chloroform extract led to the identification of panicudine (6-hydroxy-11-deoxy-13 dehydrohetisane).

Studies of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of panicudine on the tested organisms showed that the lowest MIC and the MBC were demonstrated against Salmonella paratyphi, Bacillus subtilis and Salmonella typhi and the highest MIC and MBC were against Staphylococcus aureus.

Derita.MG et al.,[19] studied on the antifungal activity of polygodial isolated from *polygonum acuminatum* and of the biotransformation product. The four extracts of *P. acumintaum* were evaluated against a panel of human opportunistic and pathogenic fungi.

Results showed that all yeasts and dermatophytes, were sensitive to P. acuminatum extracts DCM extract displayed the broadest spectrum of action, inhibiting 6/9 fungi tested The bioguided fractionation of the DCM extract led to the isolation of polygodial which on biotransformation by A. fumigatus led to the obtainment of decahydronaphtofuran-1-ol known as isodrimeninol. Both compounds were evaluated for antifungal activity and results showed that polygoidal possessed the strongest antifungal activity.

karuppiah Pllai Manoharan[20] investigated on isolation, characterization and evaluation of *Polygonum bistorta* for anticancer potential, evaluated the chloroform and hexane fractions. Both the chloroform and hexane fractions showed moderate to very good activity against P388, HL60 and LL2 cancer cell lines.

Investigations on the chemical constituents of this plant, revealed the presence of a three new [24(E)-Ethylidenecycloartanone, 24 (E)-Ethylidenecycloartan-3α-ol, 24,31-Epoxy-24ethylcycloartan-3α-o**l**] and two known (Cycloartane-3,24-dione,24Methylenecycloartanone)cycloartane-type triterpenoids. Other known compounds, β-sitosterol, friedelin**)**, γ-sitosterol, 3β-friedelinol and β-sitosterone were also isolated. All the cycloartane-type compounds, friedelin and β-sitosterone were reported for the first time from this plant.

P. Sivakumar et al.,[21] conducted phytochemical studies on *polygonum glabrum***.** The phytochemical screenings of chloroform extract shown the presence of

chemical compounds like alkaloids, glycosides, carbohydrates and flavonoids. .The chromatographic studies shows various spots (Paper/Hptlc) with chloroform extract confirmed the presence of alkaloidal contents in the plant.

Minal Jani et al.,[22] investigated on the qualitative phytochemical evaluation and antibacterial screening of *hygrophilla auriculata, polygonum glabrum*, *lemmna gibba*. The Phytochemical screening indicated the presence phenolic compounds, tannins and alkaloids in leaves of *polygonum glabrum* and various extracts of the plants have shown significant antibacterial activity.

D. Jamal Basha et al.,[23] studied hepatoprotective activity of root stocks of polygonum glabrum willd. The methanol extract of *Polygonum glabrum* produced significant hepatoprotective effect by decreasing the activity of serum enzymes, and bilirubin. Results suggested that MEPG could protect from paracetamol-induced lipid peroxidation due to the presence of phenolic compounds and they eliminating the deleterious effects of toxic metabolites from paracetamol.

Jamal Basha D et al.,[24] evaluated the antipyretic activity of the Methanolic extracts of root stocks of *polygonum glabrum willd*. The methanol extract at a dose of 200mg/kg and 400 mg/kg were evaluated for the activity in wister strain albino rats. The result showed a significant dose dependent anti pyretic effect in yeast induced elevation of body temperature in experimental rats. The Phytochemical screening of the extract revealed the presence of alkaloids flavonoids and phenolic compounds.

Bhupinder Sing et al.,[25] conducted the anti-inflammatory studies on a hot water decoction and an ethanol extract of the stems of *Polygonum glabrum***.** *The results* revealed that both the hot water decoction and the 95% ethanol extract of *P. glabrum* possessed significant anti-inflammatoryactivity against carrageenan-induced pedal oedema in rats. The extracts were more effective parenterally than by oral administration.

Queen Rosary Sheela X et al.,[26] conducted pharmacognostic and Phytochemical investigation on leaves of *polygonum barbatum*. Transverse section of *Polygonum barbatum* leaves shows that the midrib consists of broad boat shaped abaxial and broad conical adaxial side. The lamina shows the presence of glandular trichome, paracytic stomata and drused calcium oxalate crystals. The phytochemical screening of the extracts confirmed the presence of alkaloids, carbohydrates, saponins and flavonoids.

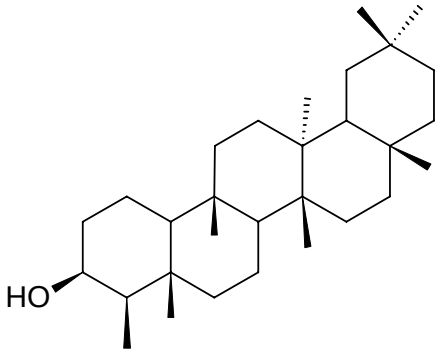
Bashir Ahmad Chaudhry et al.,[27] studied the biological activities of aerial parts of *polygonum barbatum*. The aerial parts were extracted successively with dichloromethane and methanol and were subjected to a battery of biological tests. Results revealed that the methanolic extract posses cholinergic, antifungal activity. Dichloromethane extract posses spasmolytic, brine shrimp toxicity.

X. Queen Rosary Sheela et al.,[28] conducted the antimicrobial screening of ethanolic leaf extract of *Polygonum barbatum.* The extract was assessed on ten micro organisms which include four gram positive, four gram negative bacteria and two funguses. The ethanolic leaf extract showed considerable activity on both the bacteria and fungi with Inhibition Zone Diameter (IZD) ranging from 14.17 mm to 31.45 mm. The results of research shown that the ethanolic extract of *polygonun barbatum* is a broad spectrum agent which can be used against gram positive, gram negative and also fungi.

Mohammad Abdul Mazid et al.,[29] investigated potential antitumor activity of two polygonum species. The extracts of *P. barbatum* var. *barbata* and *P. stagninum* were assessed for potential antitumor properties using the potato disc assay. All extracts showed a considerable level of potential antitumor activity. The petroleum ether extract of *P. barbatum* var. *barbata* and *n*-hexane and ethyl acetate extracts of *P. stagninum*, having IC50 values of 290, 200 and 180 μg/disc, respectively, were the most active among the extracts. Overall, the extracts of *P. stagninum* showed better antitumor activity potential than the extracts of *P. barbatum* var. *barbata*

M. Abdul Mazid et al.,[30] *investigated on the antinocicetive, anti-inflammatory and diuretic properties* of extracts of *polygonum barbatum (l.)* at the doses of 200 and 400 mg/kg body weight,were evaluated in mice/rat models using, respectively, the acetic-acid-induced writhing method,the carrageenan-induced edema test and the Lipschitz method.

Results showed that the petroleum ether extract at the dose of 400 mg/kg body weight displayed the highest levels of analgesic and anti-inflammatory activity. All extracts increased urine volume in a dose-dependent manner, and the ethyl acetate extract showed a significant level of diuresis comparable to that of the standard diuretic agent furosemide



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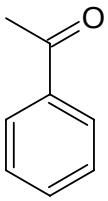

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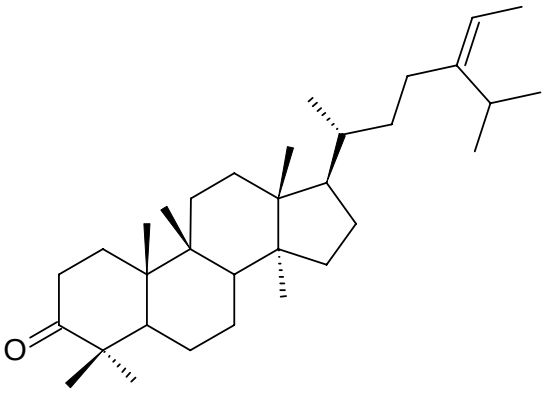
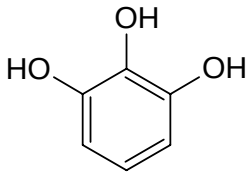
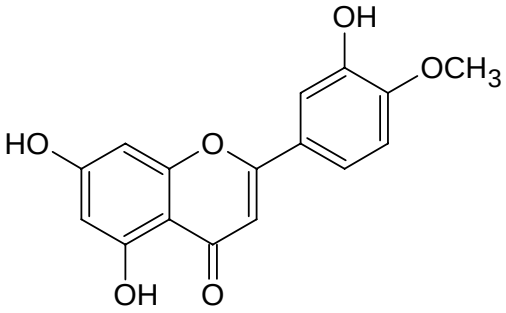


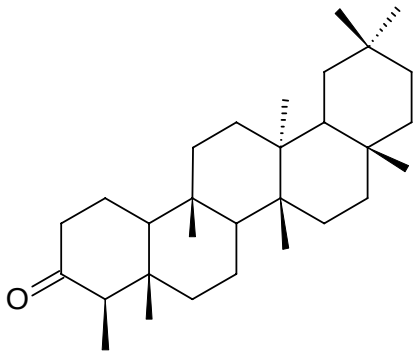
24(E)-ethylidenecycloartanone



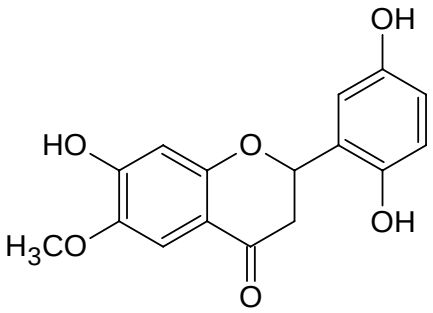
benzene-1,2,3-triol



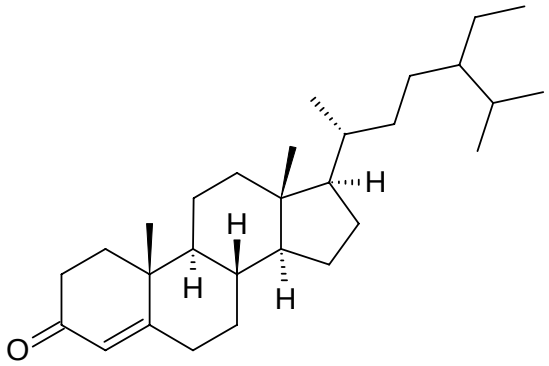
5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-chromen-4-one



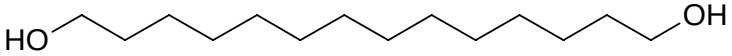
friedelin



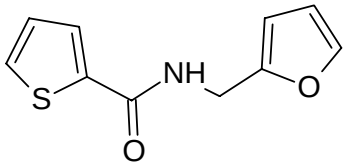
2-(2,5-dihydroxyphenyl)-7-hydroxy-6-methoxy-2,3-dihydro-4*H*-chromen-4-one



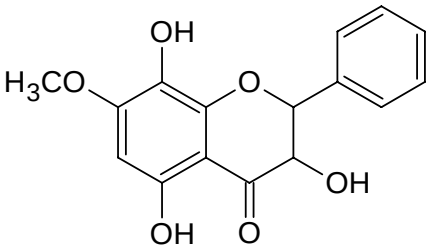
sitosterone



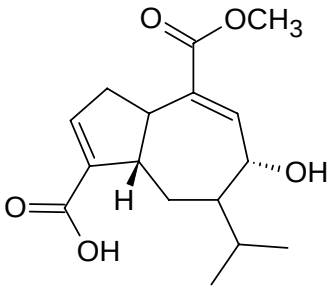
tetradecane-1,14-diol



N-(furan-2-ylmethyl)thiophene-2-carboxamide



3,5,8-trihydroxy-7-methoxy-2-phenyl-2,3-dihydro-4*H*-chromen-4-one



(6*R*)-6-hydroxy-4-(methoxycarbonyl)-7-(propan-2-yl)-3,3a,6,7,8,8a-hexahydroazulene-1-carboxylic acid

AIM OF THE PRESENT STUDY

In view of the pharmacological, biological properties and chemical constituents of plant *POLYGONUM GLABRUM* WILLD; it was decided to study the aerial parts, which is widely used as indigenous medicine.

The aim of this dissertation work was divided in to the following region.

- Preparation of crude extract by hot percolation method using various solvents of increasing polarity.
- Preliminary screening of crude extracts obtained after solvent extraction and partial purification by column chromatography and Phytochemical test analysis.
- Isolation and purification of selective phytoconstituents
- Characterization of purified compound by physical, chemical and spectral data.
- To study the pharmacological and antimicrobial activities of crude extracts.

PLANT DESCRIPTION[31-34]

BOTANICAL NAME : ***Polygonum glabrum* willd**

VERNACULAR NAMES:

Sanskrit	:	Rakta rohita
Tamil	:	Actalaree, Atalari
Malayalam	:	Attarali, *Sckoranna mudela mucca*
English	:	Poligonum
Hindi	:	Raktarohita
Assam	:	*Patharua*
Kannada	:	Neeru kangilu
Bengali	:	Bihagni

SCIENTIFIC CLASSIFICATION:

Kingdom	:	Plantae
Division	:	Magnoliaphytina
Class	:	Magnoliopsida
Order	:	Caryophyllales
Family	:	Polygonaceae
Genus	:	Polygonum
Subspecies	:	Glandulosissum

The polygonum glabrum belongs to family polygonaceae, a genus of herbs, shrubs or trees comprising about 30 genera and 1,000 species. Polygonum glabrum is commonly known as common marsh buckwheat. It is an erect herbaceous plant grows in moist habitat widely in the river banks, water logged areas and ponds, distributed in all over India. The plant was also found in Africa, Asia, and Australia.

Habitat	:	Marshy Land
Plant Type	:	Herb
Foliage	:	Evergreen
Plant Height	:	Very Small (0-5m)
Actual Height		
Maximum	:	1.50 Meter
Altitude (m)	:	000 – 2000
Plant feature	:	Erect, Forest Plant, Hill Side Plant
Plant Utilities	:	Medicinal Plant, Vegetable Crop
Season	:	Annual
Roots	:	Shallow roots, Tap roots appear at nodes
Type of stem	:	Non Woody, Swollen nodes
Leaf Type	:	Lanceolate, Linear, Margin entire, Veins numerous
Leaf Arrangement	:	Alternate
Leaf Colour	:	Green
Type of fruit	:	True fruit
Fruit classification	:	Dry fruit, indehiscent fruit, nut, simple fruit
Fruit habit	:	Cluster
Seeds	:	One seeded
Flower colour	:	Pink, white
Flower type	:	Hermaphrodite
Characteristics	:	Cluster flowers

ETHINO MEDICAL USES AND PROPERTIES

The plant has a great medicinal value.

- ✓ Root stocks are used in piles, jaundice, debility
- ✓ Seeds are used in leucorrhoea
- ✓ Leaf extract is mixed with common salt boiled and used in dysentery.
- ✓ Stem peels are burnt over affected parts of the body to treat rheumatism
- ✓ Plant pacifies vitiated pitta, inflammation, cough, asthma, pneumonia, abdominal colic, intestinal worms, muscular spasm and liver and spleen diseases

It is also used as a vegetable crop.

- ✓ The young roots and shoots are edible.
- ✓ Pungent leaves are cooked in small quantities with other vegetables.

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PHYTOCHEMICAL INVESTIGATION OF THE VARIOUS EXTRACTS OF THE *POLYGONUM GLABRUM* WILLD[35-47]

Collection of plant materials:

The details regarding the description of plant were already given. The plants of *Polygonum glabrum* willd were collected from Madurai during the months of July and identified by Dr.Stephen Lecturer, American college, Madurai. The plants were then washed with water to remove soil and other extraneous matter. The whole plant was cut into small pieces and was dried under shade for 25 days. Then the dried plant was homogenized to coarse powder and was used to different investigation

Preparation of various extracts of *Polygonum glabrum* willd using different solvents of increasing polarity.

SOLVENT EXTRACTION

Plant parts used:

Coarsely powdered shade dried aerial parts of the plant *Polygonum glabrum* willd.

Method:

Hot percolation

Materials used:

1. Soxhlet apparatus
2. Condenser
3. R. B. flask
4. Heating mantle

Chemicals used:

1. Petroleum ether A. R
2. Chloroform A. R
3. Ethanol A. R

Method of Extraction:

About 400gms of dried coarse powder was soaked into 4 liters of petroleum ether for two days. Then it was extracted first with petroleum ether ($40^oC - 60^oC$) by continuous hot percolation method using Soxhlet apparatus for 72 hrs. The petroleum ether extract was filtered and concentrated to a dry mass by distillation. A dark blackish green gressy residue with characteristic odour was obtained (14gms).

The marc left after the petroleum ether extraction was dried and subsequently extracted with chloroform for 72hrs. The chloroform extract was also filtered and concentrated to a dry mass by distillation. A blackish green gressy residue with characteristic odour was obtained (10gms).

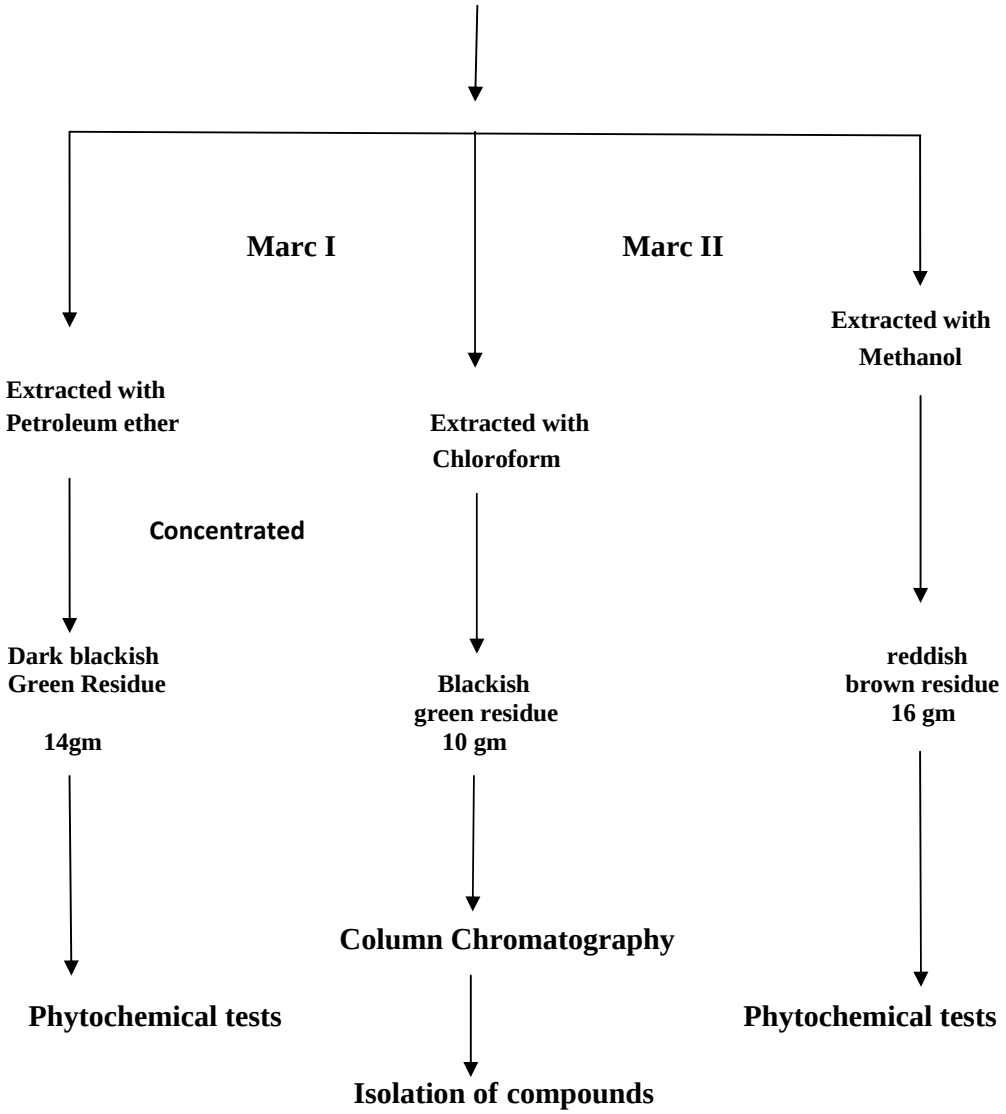
Then marc left after the chloroform extraction was dried and subsequently extracted with methanol for 72hrs. The Methanolic extract was filtered and concentrated to dry mass by distillation. A reddish brown nongreesy residue with characteristic odour was obtained (16gms).

FLOW CHART FOR VARIOUS EXTRACTION AND ISOLATION OF COMPOUNDS FROM *Polygonum glabrum* willd

SCHEMATIC DIAGRAM – I

Dry coarse material 400gm extracted by hot continuous percolation

Method using Soxhlet apparatus



PRELIMINARY QUALITATIVE CHEMICAL EVALUATION

The extracts obtained by the above methods were subjected to qualitative test for identification of various plant constituents.

1. DETECTION OF CARBOHYDRATE

★ Dissolved minimum amount of the extracts in 5ml of chloroform and filtered it. The filtrate was subjected to Molish's test to detect the presence of carbohydrate.

Molish's test

★ Filtrate was treated with 2-3drops of 1% alcoholic α – naphthol and 2ml of concentrated sulphuric acid was added along the sides of test tube. Violet coloured ring was formed at the junction of the two liquids from the pet ether, methanolic and chloroform extracts. It's showed the presents of carbohydrates.

2. DETECTION OF GLYCOSIDES

★ Small quantity of all extracts were hydrolyzed with hydrochloric acid for two hours in a water bath and the hydrosylate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

Legal's test

★ To the hydrosylate extract, 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide.

Pink to yellow colour was obtained in Methanolic and chloroform extracts showed the presence of glycosides.

Borntrager's test

★ The extracts were treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added.

Appearance of pink colour was observed in Methanolic and chloroform extracts, which showed the presence of glycosides.

3. DETECTION OF PHENOLIC COMPOUNDS

★ The extracts were dissolved in water individually and to this a few drops of ferric chloride solution were added

A violet colour was produced in methanolic extract indicating presence of phenolic compounds.

4. DETECTION OF SAPONINS

★ The extracts were diluted, with 20ml of distilled water and it was agitated in a graduated cylinder for 15minutes. A one centimeter layer of foam was produced in pet. Ether, chloroform and methanolic extracts indicating the presence of saponins.

5. DETECTION OF TANNINS

Gelatin test

★ All the extracts were dissolved separately in minimum amount of water and filtered. To the filtrate, added 1ml of 1% solution of gelatin.

None of the extracts gave white precipitate indicating the absence of tannins.

Ferric chloride test

★ The residues of all extracts were dissolved in water individually and to this a few drops of ferric chloride solution were added.

No bluish black precipitate was produced in any extracts indicating the absence of tannins.

6. DETECTION OF PROTEIN AND AMINO ACIDS

★ Small quantities of all extracts were dissolved in few ml of water and they were subjected to millon's, Biuret and Ninhydrin tests.

Millon's test

★ The above prepared extracts were treated with millon's reagent and heated.

Red colour was produced with pet. Ether, chloroform and methanolic extracts.

Biuret test

★ To the above prepared extracts equal volume of 5% sodium hydroxide and 1% copper sulphate were added.

Violet colour was produced with pet. Ether, methanolic and chloroform extracts.

Ninhydrin test

★ The above prepared extracts were treated with Ninhydrin reagent.

Blue colour was produced with chloroform, pet. Ether and methanolic extracts.

★ The above three test indicated the presence of proteins and amino respectively.

7. DETECTION OF FLAVANOIDS

Shinoda's test

★ A small quantity of the extracts, were dissolved in alcohol and to this magnesium metal followed by concentrated hydrochloric acid was added in drop wise and heated.

A magenta colour was produced only in methanolic and chloroform extracts, indicating the presence of flavonoids.

★ Small quantity of the extracts were dissolved in chloroform, added small amount of ferric chloride and potassium ferricyanide.

A deep blue colour was produced in Methanolic and chloroform extracts showed the presence of flavonoids.

8. DETECTION OF FLAVONES

- ★ With sodium, hydroxide solution, the methanolic extract gave yellow colour.
- ★ With concentrated sulphuric acid, methanolic extract gave orange colour.

Zinc, HCl Reduction test

- ★ To a small quantity of all three extracts, a pinch of zinc dust and few drops of concentrated hydrochloric acid were added. A Magenta colour was produced in methanolic extract.

Lead acetate solution test

- ★ To a small quantity of all extracts a few drops of 10% lead acetate solution was added.

  Yellow precipitate was produced in methanolic extract, indicating the presence of flavones.

- ★ From the above all tests, it is confirmed that flavones are present in Methanolic extract.

9. DETECTION ALKALOIIDS

- ★ A small quantity of the extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrate was treated with various alkaloidal reagents.

Mayer's test

- ★ All the extracts were mixed with Mayer's reagent (potassium mercuric iodide K_2Hgl_4) pale yellow precipitate was obtained with Methanolic and chloroform extracts. It's showed the presence of alkaloids.

Dragondorff's test

★ All the extracts were mixed with Dragondorff's reagent (potassium bismuth iodide), orange red precipitate was obtained in Methanolic and chloroform extracts, which indicate the presence of alkaloid.

Wagner's test

★ All extracts were mixed with Wagner's reagent (iodine in potassium iodide), reddish brown precipitate was obtained in Methanolic and chloroform extracts, which showed the presence of alkaloids.

Hager's test

★ All the extracts mixed with hager's reagent (saturated aqueous solution of picric acid). Yellow crystalline precipitate was obtained in methanolic and chloroform extracts, which showed the presence of alkaloids.

★ From the above all test, it is confirmed that alkaloids are present in Methanolic and chloroform extracts.

DATA SHOWING THE PRELIMINARY PHYTOCHEMICAL SCREENING OF THE PET. ETHER, CHLOROFORM, METHANOL EXTRACT OF *POLYGONUM GLABRUM WILLD*

TABLE.NO.1

S.NO.	CONSTITUENTS	PET.ETHER EXTRACT	CHLOROFORM EXTRACT	METHANOL EXTRACT
1	CARBOHYDRATE	+	+	+
2	GLYCOSIDES	+	+	+
3	ALKALOIDS	-	+	+
4	FLAVANOIDS	-	+	+
5	FLAVONES	-	-	+
6	PHENOLIC COMPOUNDS	-	-	+
7	PROTEINS& AMINO ACIDS	+	+	+
8	TANNIINS	-	-	-
9	SAPONINS	+	+	+

+ → indicates positive test results
- → indicates negative test results

These crude extracts were also investigated for the exhibition of some selective pharmacological activities.

BEHAVIOUR OF POWDER

Coarsely powdered leaf and stem bark of Polygonum glabrum willd was treated with various reagents.

TABLE NO.2

REAGENTS	COLOUR/PPT	CONSTITUENT
Powder as such	Brownish Green	
Powder + conc H_2SO_4	Reddish brown	Steroids present
Powder + aqs Ferric chloride	No black colour	Tannins absent
Powder + Iodine solution	No blue colour	No starch present
Powder + aqs Mercuric chloride	Brown colour	Alkaloids present
Powder + Picric acid	Yellow colour	Alkaloids present
Powder + MgHCL	Light yellow colour	Flavonoids present
Powder +aqs Silver nitrate	Precipitates	Protein present

ISOLATION, PURIFICATION AND IDENTIFICATION OF THE CONSTITUENTS

Based on the evidence of crude extract, 7 gm of Chloroform extract was chromatographed over about 300 gm of silica gel column. The solvent used were petroleum ether, benzene, chloroform, ethyl acetate, methanol and their mixtures is in the order of increasing polarity.

The column was packed by using the suspension of silica gel in petroleum ether.

Each 50ml of the elutes were collected and concentrated. Each fraction was tested for the presence of various constituents and checked on TLC for number and type of constituents.

Details of Column Chromatography

Adsorbent	:	Silica Gel G (100 – 200 mesh)
Eluent	:	Petroleum ether –methanol – Chloroform (In Graduation)
Diameter of Column	:	3 cm
Length of Column packing	:	45 cm
Amount of chloroform Extracts used	:	7 gm
Rate of elution	:	25 drops / min

Preparation of thin layer chromatography plate

About 30 gm of silica gel G was weighed, and it was shaken with 100 ml of distilled water to form a homogenous suspension. This suspension was poured into a TLC applicator, which was adjusted to 0.25 mm thickness.

The plates were kept in the hot air oven at 100°C for ½ hour to activate the silica Gel G. the plates were then stored in a dry atmosphere and used whenever required.

By using capillary tube, the fractions were spotted on TLC plates and the chromatogram was run in different solvent system. The compounds were developed related to their affinity towards different solvent system.

The different spot developed in each solvent system were identified in the iodine chamber and calculated the R_f Value.

$$R_F \text{ Value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

DATA SHOWING THE COLOUMN CHROMATOGRAPHY OF CHLOROFORM EXTRACT OF *POLYGONUM GLABRUM* WILLD

TABLE.NO. 3

FRACTIONS	ELUTES	COLOUR OF RESIDUE
1 – 4	Hexane 200 ml	Colourless
5 – 8	Hexane : benzene [40: 10,35: 15,30: 20,25: 25]	Yellow
9 – 1o	Benzene 100 ml	Brownish green
11 – 13	Benzene : chloroform [40: 10,30: 20,25: 25]	Dark Brown
14 – 15	Chloroform 100 ml	Green
16 – 19	Chloroform : ethyl acetate [40: 10,35: 15,30: 20,25: 25]	Light green
20 – 21	Ethyl acetate 100 ml	Light green
22 – 24	Ethyl acetate : methanol [40: 10,30: 20,25: 25]	Light green
25 – 28	Methanol 200 ml	Light green

DATA SHOWING TLC ANALYSIS OF

CHLOROFORM EXTRACT

TABLE NO. 4

FRACTIONS	ELUTE SYSTEM	R_f VALUE
PG 1	ETHYLACETATE : HEXANE (6 :4)	0.72
PG 2	HEXANE : ETHYLACETATE (6 : 4)	0.78
PG 3	HEXANE : ETHYLACETATE (7 : 3)	0.75

IDENTIFICATION OF ISOLATED COMPOUNDS

I.COMPOUND PG 1

The compound was isolated from the dried aerial part of ***Polygonum glabrum Willd***

1. Physical Examination:

Colour	:	green
State	:	Semi solid
Yield obtained	:	100mg
Solubility	:	Absolute alcohol, Chloroform
Melting point	:	$200 – 220^0$ C

2. T.L.C system

Adsorbent	:	Silica gel
Solvent system	:	Ethyl acetate : Hexane (6:4)
Identification	:	Iodine Chamber
R_f value	:	0.72

3. CHEMICAL TEST:

❖ **Detection of *Flavonoids***

a) SHINODA'S TEST:

A small quantity of the compound - was dissolved in alcohol and to this magnesium metal followed by concentrated HCL was added in drop wise and heated. Magenta colour was produced indicating the presence of Flavonoids

b) Small quantity of the extracts was dissolved in chloroform, added small amount of ferric chloride and potassium ferricyanide.

A deep blue colour was produced indicating the presence of flavonoids..

4) IR ANALYSIS

Media: KBr

The spectrum attached. IR peaks and the groups assigned are shown in the table.

COMPOUND PG 1

TABLE NO. 5

IR SPECTRUM DATA

S.NO	FREQUENCY CM^{-1}	GROUPS ASSIGNED
1	3345.50	O-H Stretching
2	2921.69	C-H Stretching
3	1651.70	C=O Stretching
4	1541.71	C=C Stretching
5	673.35	C-H Bending

5) ^{1}H NMR spectra of compounds:

^{1}H NMR was taken using $CDCl_3$ in 300mHZ. TMS as standard as shown in the table and the spectrum was attached.

TABLE. 6

^{1}H NMR SPECTRUM DATA

SNO	SIGNAL σ VALUES (PPM)	GROUPS ASSIGNED
1	0.896	due to CH3 proton
2	1.233	due to alcoholic hydroxyl proton
3	2.3328	due to proton of saturated carbon not attached to any hetero atom or unsaturated carbon atom
4	7.398	Due to aromatic proton

II.COMPOUND PG 2

The compound was isolated from the dried aerial parts of ***Polygonum glabrum* willd**.

1. Physical Examination:

Colour	:	Brown
State	:	Semi solid
Yield obtained	:	120mg
Solubility	:	Absolute alcohol
Melting point	:	170 - 180^0 C

2. T.L.C SYSTEM

Adsorbent	:	Silica gel
Solvent system	:	Hexane : Ethyl acetate (6:4)
Identification	:	Iodine Chamber
R_f value	:	0.78

3. CHEMICAL TEST:

❖ **Detection of *Flavonoids***

a) SHINODA'S TEST:

A small quantity of the compound - was dissolved in alcohol and to this magnesium metal followed by concentrated HCL was added in drop wise and heated. Magenta colour was produced indicating the presence of Flavonoids

b) Small quantity of the extracts was dissolved in chloroform, added small amount of ferric chloride and potassium ferricyanide.

A deep blue colour was produced indicating the presence of flavonoids.

4) IR ANALYSIS

Media: KBr

The spectrum attached. IR peaks and the groups assigned are shown in the table.

COMPOUND PG 2

TABLE NO. 7

IR SPECTRUM DATA

S.NO	FREQUENCY(CM^{-1})	GROUPS ASSIGNED
1	3447.99	O-H Stretching
2	2919.70	C-H Stretching
3	1630.25	C=O Stretching
4	1454.54	C-H Bending
5	1163.63	C-O-C Stretching
6	675.09	C-H Bending

5) ^{1}H NMR spectra of compounds:

^{1}H NMR was taken using CDCl3 300mHZ. TMS as standard as shown in the table and the spectrum was attached.

^{1}H NMR SPECTRUM DATA

TABLE NO. 8

S.NO	SIGNAL(σ)VALUES PPM	GROUPS ASSIGNED
1	0.827	due to CH3 proton
2	1.568	due to alcoholic hydroxyl proton
3	3.688	due to proton of saturated carbon not attached to any hetero atom or unsaturated carbon atom
4	7.262	due to aromatic proton

III.COMPOUND PG 3

The compound was isolated from the dried aerial parts of ***Polygonum glabrum*** **willd.**

1. Physical Examination:

Colour	:	Light green
State	:	Semi solid
Yield obtained	:	50mg
Solubility	:	Chloroform
Melting point	:	160 - 170^{0}C

2. T.L.C SYSTEM

Adsorbent	:	Silica gel
Solvent system	:	Hexane : Ethyl acetate (7: 3)
Identification	:	Iodine Chamber
R_f value	:	0.75

3. CHEMICAL TEST:

❖ **Detection of *Flavonoids***

a) SHINODA'S TEST:

A small quantity of the compound - was dissolved in alcohol and to this magnesium metal followed by concentrated HCL was added in drop wise and heated. Magenta colour was produced indicating the presence of Flavonoids

b) Small quantity of the extracts was dissolved in chloroform, added small amount of ferric chloride and potassium ferricyanide.

A deep blue colour was produced indicating the presence of flavonoids.

4) IR ANALYSIS

Media: KBr

The spectrum attached. IR peaks and the groups assigned are shown in the table.

COMPOUND PG 3

TABLE NO. 9

IR SPECTRUM DATA

S.NO	FREQUENCY(CM^{-1})	GROUPS ASSIGNED
1	3421.94	O-H Stretching
2	2919.70	C-H Stretching
3	1560.36	C=C Stretching
4	1426.57	C-H Bending
5	1211.18	C-O-C Stretching
6	665.73	C-H Bending

5) ^{1}H NMR spectra of compounds:

^{1}H NMR was taken using CDCl3 300mHZ. TMS as standard as shown in the table and the spectrum was attached.

^{1}H NMR SPECTRUM DATA

TABLE NO. 10

S.NO	SIGNAL(σ)VALUES PPM	GROUPS ASSIGNED
1	0.863	due to CH3 proton
2	1.284	due to alcoholic hydroxyl proton
3	3.877	due to proton of saturated carbon not attached to any hetero atom or unsaturated carbon atom
4	4.817	due to vinylic proton
5	7.262	due to aromatic proton

HEPATOPROTECTIVE ACTIVITY OF *POLYGONUM GLABRUM* AGAINST RIFAMPICIN AND ISONIAZID -INDUCED TOXICITY IN WISTAR RATS[48-58]

INTRODUCTION

Isoniazid, although a highly effective drug in the treatment of tuberculosis, is well known for its hepatotoxicity. The risk of severe hepatotoxicity caused by Isoniazid is approximately 1–2% of patients and 20% of patients are associated with liver enzyme elevations in plasma. Despite the undefined mechanism of Isoniazid hepatotoxicity, hydrazine and acetyl hydrazine are regarded as the main toxic metabolites of Isoniazid. It is highly suggested that these two bioactive metabolites are produced by a series of enzymes including cytochrome P450 and could induce oxidative stress to cause hepatotoxicity. In particular, CYP 2E1 is reportedly involved in Isoniazid-induced hepatotoxicity in humans[. Animals, microsomes and HepG2 cells.

Since oxidative stress induced by CYP 2E1 was regarded as the major mechanism of Isoniazid hepatotoxicity, intracellular glutathione (GSH) enhancers and reactive oxygen species (ROS) scavengers were used as a potent anti-hepatotoxic drugs against Isoniazid toxicity. For example, 'thiol' compounds, such as *N*-acetylcysteine (NAC), effectively inhibited the Isoniazid hepatotoxicity in rat by supplying the intracellular GSH content. Rifampicin (RIF), a powerful inducer of mixed-function oxidase, increases the hepatotoxicity of Isoniazid (INH) by enhancing the production of toxic metabolites from acetyl hydrazine. Rats show a similar genetically determined acetyl transferase activity as in humans and are more sensitive to Isoniazid (INH)-induced hepatotoxicity due to a high amidase activity, which results in release of large amount of acetyl hydrazine, which induces hepatotoxicity. Anti-tubercular drugs mediated oxidative damage is generally attributed to the formation of free radicals, which act as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane. Alterations of various cellular defense mechanisms consisting of enzymatic and non-enzymatic components have been reported in Rifampicin (RIF) and Isoniazid (INH) induced hepatoxicity.

Since all the drugs used in the treatment of tuberculosis are shown to have hepatotoxic effects, studies have been performed to prevent or reduce the toxicity by

the use of natural herbal drugs and/or synthetic compounds, without interfering with the therapeutic action of the drugs.

Garlic, Silymarin, N-acetylcysteine and several other herbal drugs are proved to have such effects. It is of importance to note that the inhibition of CYTP450 2E1 and antioxidant actions seem to be the common mechanism of action of herbal drugs.

The present study was made to investigate the protective actions of Methanolic Extract of *polygonum glabrum* (MEPG) against hepatotoxicity caused by rifampicin-Isoniazid.

MATERIALS AND METHODS

Chemicals

Total albumin, Total protein, Aspartate transaminase (AST), Alanine Transaminase (ALT) and Alkaline phosphatase (ALP) were assayed by using kits from Ranbaxy Diagnostic, New Delhi. All the drugs, chemicals and reagents used for biochemical estimation were purchased from Sigma-Aldrich, USA.

Animals

Male Wistar albino rats, weighing about 150 – 200 g were obtained from Institute Animal Center and used in the experiments. The protocol was approved by the Institute's Animal Ethical Committee. Animals were kept in the animal house at an ambient temperature of 25°C and 45-55% relative humidity, with 12 h each of dark and light cycles. Animals were fed pellet diet and water *ad-libitum*.

Induction of experimental hepatotoxicity

Rifampicin and Isoniazid solution were prepared separately in sterile distilled water. Rats were treated with Isoniazid (100 mg/kg, orally) and co-administered with rifampicin (100 mg/kg, orally) for 21 days. In order to study the effect of Methanolic extract of *polygonum glabrum* (MEPG) in rats, 200 and 400 mg/kg, orally were used. Silymarin (2.5 mg/kg orally.) was used as a standard drug in this study.

TREATMENT PROTOCOL

Rats were divided into five groups each having six animals.

Group1 Served as normal control group received 10ml/kg normal saline.

Group2 Served as toxic control group received Rifampicin and Isoniazid 100mg/kg administered orally.

Group3 Served as standard group received Silymarin 2.5 mg/kg administered orally.

Group4 Served as extract (MEPG) treatment groups received 200 mg/kg administered orally.

Group5 Served as extract (MEPG) treatment groups received 400 mg/kg administered orally.

Weights of these rats were monitored sequentially in control and experimental animals for a period of 21 days.

Biochemical estimation

Rats were sacrificed 1 h after administration of drug on day 21.The blood was collected by retro-orbital artery puncture. Blood samples were centrifuged for 10 min at 3000 rpm to separate the serum. AST, ALT, ALP, Total Protein and albumin levels were estimated from the serum by using standard kits [20].

Statistical analysis

The results are expressed as mean ± SEM. The evaluation of the data was done using one way ANOVA followed by Newman – Keul's multiple range tests. Difference below $p<0.05$ implied significance.

TABLE NO. 11

EFFECT OF MEPG ON SERUM ENZYMES

GROUPS	TOTAL PROTEIN (g/dl)	TOTAL ALBUMIN (g/dl)	AST (u/l)	ALT (u/l)	ALP (u/l)
G1	9.20±0.88	5.88±0.45	142.22±5.88	77.42±4.25	122.42±5.15
G2	4.16±0.22*a	2.26±0.29*a	260.30±7.78*a	180.45±5.92*a	289.62±7.62*a
G3	7.98±0.76*b	4.35±0.30*b	178.12±5.60*b	85.10±3.77*b	223.88±4.38*b
G4	6.86±0.42*b	3.88±0.26*b	208.12±7.12*b	120.23±4.27*b	260.48±5.12*b
G5	7.32±0.34*b	4.12±0.40*b	194.22±6.22*b	106.55±3.98*b	248.56±4.75*b

All values are expressed as Mean ± SEM (n=6).

- Values are expressed as Mean ± SEM.
- Values were find out by using ONE WAY ANOVA followed by Newman Keul's multiple range tests.
- * (a) values were significantly different from normal control at $p<0.01$.
- * (b) Values were significantly different from toxic control at $p<0.01$.

RESULTS

Effect of *polygonum glabrum* on the serum AST levels:

Results showed a significantly increased ($p<0.01$)level of serum AST in rats of G2 as compared to G1 (control) due to Rifampicin (RIF) and Isoniazid (INH), but these levels were significantly reduced ($p<0.01$) in rats of G3, G4 and G5 treated with Silymarin and *polygonum glabrum at a* dose of 200 and 400mg/kg..

Effect of *polygonum glabrum* on the serum ALT levels:

It is found a significantly increased ($p<0.01$) level of serum ALT in rats of G2 as compared to G1 (control) due to Rifampicin (RIF) and Isoniazid (INH), but these levels were significantly reduced ($p<0.01$) in rats of G3, G4 and G5 treated with Silymarin and *polygonum glabrum at a* dose of 200 and 400mg/kg..

Effect of *polygonum glabrum* on the serum ALP levels:

It is observed that Rifampicin (RIF) and Isoniazid (INH) caused significantly increased ($p<0.01$) level of serum ALP in rats of G2 as compared to G1 (control) due to Rifampicin (RIF) and Isoniazid (INH), but these levels were significantly reduced ($p<0.01$) in rats of G3, G4 and G5 treated with Silymarin and *polygonum glabrum at a* dose of 200 and 400mg/kg..

Effect of *polygonum glabrum* on the serum total protein and total albumin levels:

The results showed that Rifampicin (RIF) and Isoniazid (INH) caused significantly decreased ($p<0.05$) level of serum total protein and total albumin in rats of G2 as compared to G1 (control) due to Rifampicin (RIF) and Isoniazid (INH), but these levels were significantly increased ($p<0.01$) in rats of G3, G4 and G5 treated with Silymarin and *polygonum glabrum at a* dose of 200 and 400mg/kg.

DISCUSSION

Rifampicin (RIF) and Isoniazid (INH) are the most important first line drugs, used for the treatment of tuberculosis. Isoniazid (INH) can cause hepatotoxicity in 20% of patients and is usually associated with an inflammatory response. Rifampicin (RIF) and Isoniazid (INH) are reported to induce hepatotoxicity judged by elevated serum AST, ALT, ALP and total bilirubin levels, presence of focal hepatocytic necrosis and portal triaditis. Plant-derived antioxidants such as Vitamin E, Vitamin C, polyphenol including phenolic acids, phenolic diterpenes, flavonoids, catechins, procyanidins, and anthocyanins are being increasingly suggested as important dietary factors. Supplementation with berry juice flavones from skullcap, catechins from green tea, anthocyanins from chokeberry, and condensed tannins from faba beans are indeed protective of oxidative stress indices in rats.

The protective action of antioxidants is usually due to the inhibition of free radical chain reaction and the resultant prevention of peroxidative deterioration of structural lipids in membranous organelles. Circulating antioxidants mainly vitamin C and vitamin E and tissue enzymatic and non-enzymatic such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) play important role in alleviating tissue damage due to the formation of free radicals. In this study, we observed the hepatoprotective effect of *polygonum glabrum* in Rifampicin (RIF) and Isoniazid (INH) induced hepatotoxicity in rats. A significant elevation was observed in the levels of serum AST, ALT, ALP and significant decrease level total protein and total albumin in G2 which received Rifampicin (RIF) and Isoniazid (INH) as compared to G1 rats who received normal saline.

Elevated levels of these parameters in serum are presumptive markers of hepatotoxic lesions in the liver. Co-administration of Silymarin and low & high dose of (200 & 400mg/kg) *polygonum glabrum* Methanolic extract with INH and RIF Rifampicin (RIF) and Isoniazid (INH) in G3, G4 and G5, maintained the levels of AST, ALT, ALP, and serum total Protein and Total albumin towards normalcy as compared to G2 rats. This was most likely due to the anti oxidant effect of *polygonum glabrum* constituents. While in G5 high dose *polygonum glabrum*

showed a significant recovery towards normal, this result shows hepatoprotection after a both dose of *polygonum glabrum extract* in experimentally drug induced hepatitis (DIH) in rats. In this study Flavonoids in *polygonum glabrum* might have a role in the recovery in Rifampicin (RIF) and Isoniazid (INH) induced hepatotoxicity in rats.

ANALGESIC ACTIVITY OF VARIOUS EXTRACTS OF *POLYGONUM GLABRUM* WILLD

Analgesic activity of various extracts of *Polygonum glabrum* was evaluated by acetic acid induced writhing reflux in mice. Painful reaction in animals may be produced by the chemicals such as phenylquinone, bradykinin etc. Like that, acetic acid pain reaction which is characterized as a writhing response. Construction of abdomen, turning of trunk (twist) and extension of hind legs are taken as reaction to chemically induced pain. Analgesics (both narcotic and non-narcotic) inhibit writhing response.

REQUIREMENTS:

Animal : Swiss albino mice (20-25g) either sex

Drugs and chemicals : Diclofenac sodium (standard),

Acetic acid (1%v/v), petroleum ether and Methanolic extract of *Polygonum glabrum*

METHOD:

TREATMENT PROTOCOL

Group-1 Treated as normal control received 10ml/kg of normal saline through orally.

Group-2 Treated as Standard control received 10mg/kg of diclofenac sodium through Intraperitonealy.

Group-3 Treated as treatment control received 200mg/kg of petroleum ether extract of *Polygonum glabrum* suspended with 2ml of 1% CMC administered through orally.

Group-4 Treated as treatment control received 200mg/kg of Methanolic extract of *Polygonum glabrum* suspended with 2ml of 1% CMC administered through orally.

Both the extracts were administered one hour prior to the acetic acid administration. Note the onset on writhing. Record the numbers of abdominal contractions, trunk twist and extension of hind limbs as well as the number of animals showing such response during a period of 10 minutes were noted.

STATISTICS:

Data are expressed as mean ± SEM; data analyzed by one way ANOVA followed by Newman's keul's multiple range tests to determine the significance of the difference between the control group and rats treated with the extracts.

* Values were considered significant at $P < 0.01$.

TABLE No.12

ANALGESIC ACTIVITY OF VARIOUS EXTRACTS OF *POLYGONUM GLABRUM* BY ACETIC ACID INDUCED WRITHING REFLUX IN MICE

Treatment	Dose (mg/kg)	No. of writhing	% reduction in reaction time
Group I Normal saline	Inject 1%v/v acetic acid 1ml/100g of body weight	39.5±4.6	-
Group II Std	10mg/kg I.P.Diclofenac sodium	6.4±1.22	83.79%**
Group III Pet. Ether extract	200mg/kg Administered through orally.	28.6±3.2	27.59%
Group IV Methanolic extract	200mg/kg Administered through orally	11.3±2.0	71.39%**

Values are expressed as mean±SEM

Values were find out by using one-way ANOVA followed by Newman's keuls multiple range tests.

** Values were considered significant at P< 0.01.

RESULTS

The table values show that analgesic activity of various extracts of polygonum glabrum by acetic acid induced writhing reflex. The results reveals that petroleum ether extract does not possess significant analgesic activity where as Methanolic extract possess significant analgesic activity at $p<0.01$.

ANTIMICROBIAL STUDIES[59-61]

INTRODUCTION

Antimicrobial drugs are the greatest contribution of the present century to therapeutics, their importance is magnified in developing countries were infective disease predominats. Drug in this class differ from all others in that their designed to inhibit or kill the infecting organism and to have no or minimal effect on the recipients.

In vitro antimicrobial activity

Antimicrobial activity is measured in order to determine.

1. The potency of an antimicrobial agent in solution
2. Its concentration in body fluids or tissues
3. The sensitivity of the given micro organism to know concentration of the drug

Factors affecting antimicrobial activity

1. pH of the environment
2. Components of medium
3. Stability of drug
4. Size of inoculums
5. Length of incubation
6. Metabolic activity of micro organism

Organism used

Gram +ve microorganism

Staphylococcus aureus

Gram –ve microorganism

Pseudomonas aurogenosa

Fungi microbials

Candida albicans

Media used

Muller-Hinton agar media

Standard used

Amikacin disc

Ketoconazole

Method

Filter paper disc method

Principle

Gel diffusion

EVALUATION OF ANTIBACTERIAL ACTIVITY OF VARIOUS EXTRACTS OF *POLYGONUM GLABRUM* WILLD

They have relatively broad spectrum of action and are effective against Gram positive and Gram negative organism.

Bacteria

Bacteria are unicellular microscopic prokaryotic organism lacking chlorophyll. They are found in animals, plant, soil, water, atmosphere and dead organic matters. Bacteria generally vary in size from 0.5µ to 3µ.

Infection

Infection can involve in any organs or system in body. The present trend is to refer to all disease caused by living micro organism as infectious diseases.

- Bacteristatic if it inhibits the growth of bacteria
- Bactericidal if it destroys and kills the bacteria

Treatment

Chemotherapy is defined as the treatment of specific infections with chemical agents so as to destroy offending micro organism or parasites without damaging the caused tissues.

Factors of chemotherapeutics agents

- Host defense
- Source of infection
- Tissues affected

- Margin of safety
- Bacterial resistance to the agent being used

Mechanism of action of antibacterial agents

- Interference with cell wall synthesis
- Damage to the cytoplasmic membrane
- Inhibition of protein synthesis and impairment of function of the ribosome
- Interference with translation of genetic information
- Inhibition of viral enzymes

Experimental

Purpose and rationale

The in vitro antibacterial activities of the methanolic and pet. Ether extracts of *Polygonum glabrum Willd* were evaluated against staphylococcus aureus and pseudomonas aurogenosa.

Requirements

- Petridish (sterilized)
- Muller-Hinton agar media (sterilized)
- Amikacin disc (standard)
- Methanol (control)
- Methanolic and pet. Ether extracts

Organism used

Gram +ve microorganism

Staphylococcus aureus

Gram –ve microorganism

Pseudomonas aurogenosa

Method: Filter paper disc method

Principle: Gel diffusion

Composition of media: Muller-Hinton agar media

Beef extract	30gm
Peptone	17.5gm
Starch	1.5gm
Agar	17gm
Sodium hydroxide	5gm
Distilled water	1000ml
Final pH at 25°C	7.4±0.2

The beef extract, peptone, starch and agar were taken in the above proportions and dissolved up to 100ml of distilled water. The constituents were heated gently at 100°C with agitation. The pH of the medium was adjusted to 7.4 using sodium hydroxide. The pH tested using a universal indicator paper, which showed green colour at pH 7.4, and then it was transferred to boiling tubes in hot conditions and sealed with non absorbent cotton and sterilized by autoclaving at 121°C (15lbs pressure) for 15 min, poured aseptically in to sterile Petri dishes.

Conditions of the work

The entire work was done using horizontal laminar flow cabinet so as to provide aseptic condition. Before commencement of the work, air sampling was carried using a sterile Mull- Hinton agar plate and exposing in to the environment inside the cabinet. On incubation it was checked for the growth of micro organism and absence of growth confirmed the aseptic working conditions.

Inoculation of microorganism

The sterilized Muller-Hinton agar media was heated on a water bath to melt the media. When the media was luke warm, the organism was inoculated separately and poured aseptically into sterile petridishes and allowed to solidify. The standard drug Amikacin disc was placed on the media and the Whatmann No.2 filter disc (5mm diameter) were cut and filled into vials plugged with cotton. These vials were kept in hot air oven at 160°C for 30 min for sterilization. Then it was soaked in two extracts separately and evaporated to dryness and kept on the media (5mm height). One more disc immersed in methanol and kept on the media as control. It was kept in the refrigerator for one hour to facilitate uniform diffusion of the drug and later kept in the incubator for a period of 24 hours at 37°C. Observations were made for the zone of inhibition around the synthesized compounds with that of standard.

ANTI BACTERIAL ACTIVITY

STANDARD DRUG: Amikacin [5µ gm / ml]

TABLE NO.13

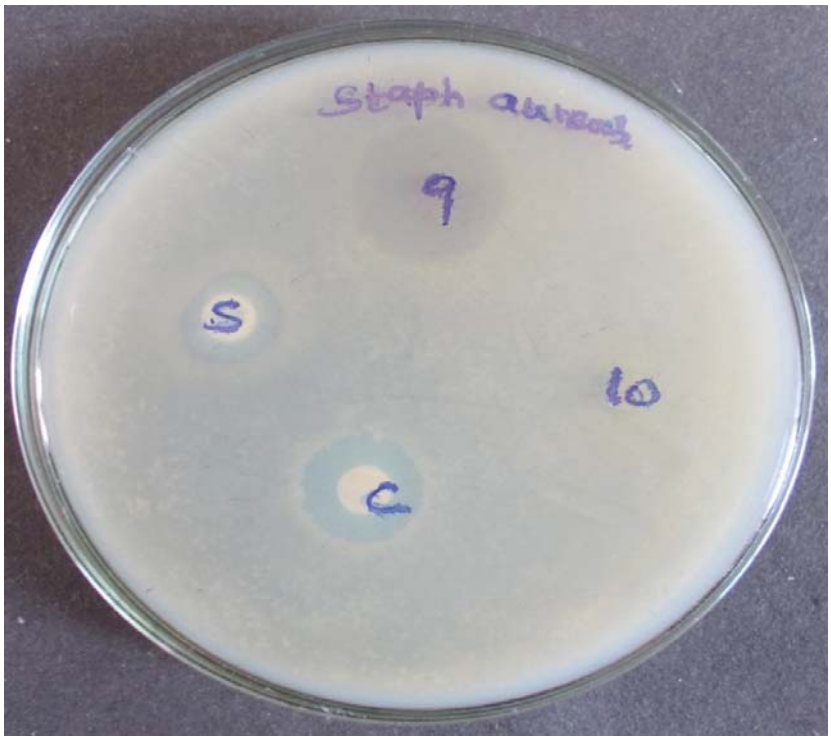
Staphylococcus Aureus

S. No	SAMPLE	ZONE OF INHIBITION
1.	Standard	17 mm
2.	Methanolic extract(9)	15 mm
3.	Pet. Ether extract(10)	4 mm

TABLE NO. 14

Pseudomonas Aurogenosa

S No	SAMPLE	ZONE OF INHIBITION
1.	Standard	14 mm
2.	Methanolic extract(9)	12 mm
3.	Pet. Ether extract(10)	6 mm


Staph aureus
9
S
10
C


P. seudomonas
9
S
10
C

EVALUATION OF ANTIFUNGAL ACTIVITY OF VARIOUS EXTRACTS OF *POLYGONUM GLABRUM WLLD*

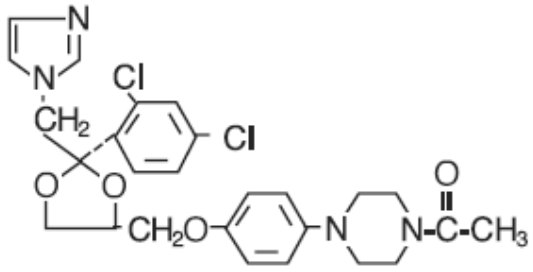
INTRODUCTION

Fungi are plant like non photosynthetic eukaryotes growing either in colony of single cell (yeast) or in filamentous multi-cellular aggregates (molds). Most fungi live as Saprophytes in soil or dead plant materials and they are very important in the mineralization of organic matter. Antifungals work by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus fungal and human cells are similar at the molecular level. This makes it more difficult to find or design drugs that target fungi without affecting human cells.

The azole antifungal drugs inhibit the enzyme lanosterol 14 α-demethylase; the enzyme necessary to convert lanosterol to ergosterol. Depletion of ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition of fungal growth.

Treatment

The standard drug used is Ketoconazole.



Ketoconazole is usually prescribed for topical infections such as athlete's foot, ringworm, candidiasis (yeast infection or thrush), and jock itch.

Experiment

Purpose and rationale

The in-vitro antifungal activity of the various extracts of *Polygonum glabrum willd* was evaluated on Candida albicans.

Requirement

- Petri-dishes (sterilized)
- Sabouraud Dextrose Agar media (sterilized)
- Ketoconazole (standard)
- Methanol (control)
- Methanolic and Pet. Ether extracts

Organisms used

Candida albicans

Method

Filter paper disc method

Principle

Gel diffusion

Composition of media

Saboured Dextrose Agar media

Glucose	-40gm
Peptone	-10gm
Agar	-15gm

Distilled water up to -1000ml

Final pH -5.4

Procedure

Glucose, peptone and agar were taken in the above proportions and dissolved up to 1000ml of distilled water. The constituents were heated gently at 100°C with agitation. The pH of the media was adjusted to 5.4. Then it was transformed to boiling tube in hot condition and sealed with non-absorbent cotton and sterilized by autoclaving at 121°C (15 lbs pressure) for 15 minutes then poured aseptically in to sterile Petri-dishes.

Microorganisms- Candida albicans

Candida albicans is seldom isolated outside the bodies of animals and is known from 58 species including wild and domestic mammals and birds. Candiasis, the disease caused by C.albicans. The cell wall polysaccharides mannan and mannan protein complexes are involved in several different interaction and mannan structure types of Candida albicans.

Working conditions

The entire work was done using horizontal laminar flow cabinet so as to provide aseptic condition before commencement of work, a sampling was carried out using a sterile Muller-Hinton agar plate and exposing it to the environment inside the cabinet. On incubation it was checked for the growth of microorganism and absence of growth confirmed the aseptic working condition

Inoculation of microorganism

For the screening of antifungal activity disc diffusion method was used. Sabouraud dextrose agar plate were prepared aseptically to get a thickness of 5-6mm.

the plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 37°C just before inoculation.

From the solid culture the clinical sample of Candida albicans were inoculated in to Sabouraud dextrose agar plates by using sterile inoculation loop and agar plates were incubated for about 24 hours, at 37°C, which show significant growth of fungi.

The temperature of the medium should not exceed about 50°C when the organisms were inoculated. The standard drug Ketoconazole (10µgm/disc) was placed on the media. The sterile Whatmann No.2 filter paper disc (5mm diameter) was soaked in two extracts (20µgm/disc) separately and evaporated to dryness and then kept on the media. One more disc immersed in methanol and kept on the media as control. The Petri dishes were incubated at 37°C for 24 hours, after placing them in refrigerator for 1 hour to facilitate uniform diffusion. Observations were made for the zone of inhibition around the extracts and with that of standard.

ANTIFUNGAL ACTIVITY

TABLE NO.15

Candida albicans

S. No	SAMPLE	ZONE OF INHIBITION
1.	Standard	14 mm
2.	Methanolic extract(9)	11 mm
3.	Pet. Ether extract(10)	7 mm